

# **EXHIBIT 1**

## Note

## Immunity, immunopathology and vaccines against HIV?

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Received 6 June 2001; accepted 28 November 2001

## Abstract

Immunity and immunopathology of HIV infections leading to AIDS are reviewed from an evolutionary point of view. Accordingly infectious agents and host defences have co-evolved to reach balanced states where virus and host survive. While HIV has not quite yet reached an optimal balance, tuberculosis (TB), leprosy, HBV, HCV in humans or lymphocytic choriomeningitis virus (LCMV) in mice have successfully established persistence. These non- or poorly-cytopathic infections infect the next host usually before or at birth while hosts are immunoincompetent. They also infect immunocompetent hosts to persist at low levels concomitant with an ongoing T and B cell immune response that is repeatedly triggered by latent or persistent infection of extralymphatic or lymphatic host cells. This infectious or infection-immunity is the basis for cellular immunoprotection by antigen activated T cells. Because we cannot imitate this infection-immunity long-term and cannot build polyspecific vaccine combinations covering all possible neutralising variants yet, vaccines against TB, leprosy, HCV and HIV only protect transiently and incompletely. © 2002 Elsevier Science Ltd. All rights reserved.

**Keywords:** HIV; Immunopathology; Vaccine; LCMV lymphocytic choriomeningitis virus; Immunosuppression

## 1. General rules of immune responses

Cytopathic infectious agents usually kill immunological low responders whereas in general high responders tend to survive. Immunological memory can improve these conditions through vaccinations. From an evolutionary point of view, immunological memory appears to be important to protect offspring during pregnancy and after birth because they are immunoincompetent [1]. Since T cells cannot be transferred from mother to offspring due to MHC polymorphism, only soluble antibodies provide a transmissible form of immunological experience [2]. Natural selection would have rendered unlikely cytopathic agents that could not be controlled efficiently by antibodies during this critical time after birth. From this point of view, high-titre antibody levels against most relevant pathogens in maternal serum at the time of pregnancy and birth is essential for the survival of the species. Maintenance of sufficient protective levels of antibody in males is also important to maintain herd immunity.

From this evolutionary point of view what then is the role of T cell memory and how can T cell and antibody memory be maintained? T cell memory is usually thought to represent increased precursor frequencies. But many experiments

have revealed that this simple concept may not satisfyingly explain the biology of protective memory. All vaccines that provide efficient protection mediate protection via protective (neutralising) antibodies not via T cells [3]. In contrast, all vaccines that do not function and do not protect lack a sufficient protective T cell immunity. We do not possess effective vaccines against tuberculosis (TB), leprosy, HIV, HCV and most parasitic infections. In all these cases, a prominent T cell immune component is essential for protection. Why does BCG not protect for long term against TB? The answer may lay in recent experiments suggesting that for protective T cell memory persistence of antigen in appropriate peripheral depots from which antigen periodically reaches draining lymph nodes or spleen may be essential. Antigen is not necessary for maintenance of increased precursor T (or B) cell frequencies [4], but is crucial for keeping T cells activated; only activated T cells are capable of emigrating into peripheral solid tissues and organs to control peripheral infections [5]. Many humans have a TB lesion in the lung that is well controlled by an ongoing immune response that is also at the same time maintained by the persistence of the granulomatous lesion. BCG infects vaccinees for a limited period of time of 1–4 years, but then is eliminated and protection fades [6]. Efficient immune protection against TB therefore seems to correlate with presence of BCG bacilli. Similarly, antibody responses are maintained by antigen in the form of

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antigen–antibody complexes on follicular and dendritic cells in germinal centres [7,8], but also periodical exposure from the outside (polioviruses or Sabin vaccines [9]) or periodical spreading from within (herpes viruses) or persistence of crippled virus in the host, e.g. measles virus [10] provide an antigen source that maintains high levels of protective antibodies. Other experiments have shown that adoptive transfer of increased precursor frequency T and B cells from primed animals by itself does not induce and maintain high antibody or activated T cells in the naïve recipient unless antigen is added [11–13]. In conclusion, therefore, protection by immunity is mediated by either pre-existent antibodies [14] or activated T cells [11,12], but not by quiescent primed B cells or memory T cells.

## 2. Lymphocytic choriomeningitis virus (LCMV) as a model for HIV

LCMV is an ambisense RNA virus [15] that may, but does not regularly, persist in a DNA form [16]. It is a non-lytic virus in its normal host the house mouse and is usually transmitted from mother to offspring during pregnancy via the placenta [17]. Infection of adult mice with LCMV causes a more or less severe T cell mediated immunopathology depending on the dose- and time of kinetics. The organ that is predominantly infected by the virus then determines the immunopathological disease: a choriomeningitis or a hepatitis or a graft-versus-host like AIDS disease [18–20]. It is particularly this last aspect that makes the LCMV model interesting for HIV studies [21]. In fact LCMV has contributed considerably in the past 10 years to a better understanding of HIV–AIDS-pathogenesis (Table 1). The immunopathologically mediated destruction of lymphohemopoietic cells and lymphatic organs is of particular interest because it much resembles what has been observed in AIDS [22,23]. LCMV infects predominantly marginal zone macrophages early after haematogenic spread. This compartment is destroyed by

Table 1  
LCMV infection, immunity and immunopathology in mice: a model for HIV–AIDS in humans

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Wide tropism and variants with different tropisms [4,15,18,41]
Non-cytopathic in mice but not in rats [36,37]
Transmission to next host transplacentally or at birth [18,42]
Persistent infection with low or high dose infections [18,37]
Ambisense-RNA virus that may persist in a DNA-form [16]
Perforin-dependent CD8 <sup>+</sup> T cell-mediated control early [43]
T cell-mediated immunopathology including immunosuppression [15,18–20,24,26,37,40,44]
T cell-exhaustion by systemic virus-spread: CD8 <sup>+</sup> T cell exhaustion [45], CD4 <sup>+</sup> T cell exhaustion [29]
Neutralizing antibodies responsible for long-term control [18,32,33,37,46]
Escape of immune surveillance by mutations: CD8 <sup>+</sup> T cell epitope escape [28], CD4 <sup>+</sup> T cell epitope escape [29a], neutralizing antibody escape [30]

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the action of virus specific CTLs [24,25]. This results in the elimination of important antigen-capturing compartments that filter haematogenically spreading antigen (including of LCMV) and causes a severe immunodeficiency. Antibody responses against a superinfecting virus such as vesicular stomatitis virus (VSV) for example are virtually impossible during this phase [26,27]. If mice are treated with an anti-CD8 antibody to eliminate their cytotoxic antiviral immuneresponse, this immunosuppression is largely avoided. But under these circumstances, LCMV is not eliminated and a carrier status is induced.

Since LCMV is a non-cytopathic virus for mice and does not cause a cell or tissue damage, the virus can easily escape immune surveillance, either at the cytotoxic T [28] or T helper cells [29], but also at the neutralising antibody level [30]. Examples have been documented for LCMV for all three cases. Cytotoxic T cell escape mutants that have exchanged one or two amino acids within a T cell epitope were discovered in LCMV infected mice expressing a transgenic T cell receptor [28]. Neutralising antibody escape variants were discovered in mice that exhibited low or no CD8 T cell responses [30]. Nevertheless, such mice eventually could eliminate the virus by day 50 or 60 and could control virus for about 3 weeks. But then virus reappeared again and this reappearing virus had escaped the neutralising antibody response against the original infecting virus. Surprisingly, in parallel, virus specific T helper cells either were exhausted or exhibited T helper cell epitope escape mutations that rendered T help ineffective [29a]. For this reason, the newly developed neutralising epitope could not induce a new neutralising antibody response in the original host [29]. In contrast, the neutralising antibody-escape variant virus was capable of inducing a good neutralising antibody response in a naïve new host. Interestingly, this neutralising antibody response against the escaped variant also induced a very strong neutralising antibody response against the original wild-type virus (this is comparable to the original antigenic sin situation in influenza virus infections [31]). Thus, LCMV causes via immunopathology an immunosuppression that enhances virus survival in the host. This process may be facilitated by the virus escaping not only cytotoxic but also T helper and neutralising antibody responses. Together, all these mechanisms enhance persistence and establishment of a virus carrier status.

## 3. Adoptive immunotherapy of virus carriers

LCMV is controlled primarily via CD8<sup>+</sup> T cells during primary infection, but virus persists at very low levels in most mice. However, in mice that cannot make antibodies the CD8<sup>+</sup> T cell-mediated control of virus may eventually break down. LCMV virus reappears in  $\mu$ MT 0/0 (IgM knockout) or CD4<sup>+</sup> T cell 0/0, or MHC class II 0/0 mice by about 100–250 days; interestingly, at that time point all primed and naïve LCMV-specific T cells have been deleted

[32]. Therefore, neutralising antibodies play a major role in preventing generalised spread of the virus that otherwise is controlled by virus-specific cytotoxic T cells. Attempts to cure virus carriers by neutralising antibodies alone have failed in all hands. However, adoptive transfer [15,33] of immune cells cured virus carriers down to undetectable levels of virus dependent on the virus strain used, the infectious dose used and the types of lymphocytes transferred. Treatment of LCMV-Armstrong carriers revealed that CD8<sup>+</sup> T cells from immune mice can cure carriers [34]. In contrast, LCMV-WE carriers were cured if 60-day immune but not if 8-day immune spleen cells were used [32,35,36]. In contrast, more recent evaluations of adoptive immunotherapy with LCMV-WE carriers showed that while CD8<sup>+</sup> T cells enhanced clearance of virus, particularly primed B cells mounting neutralising antibody responses plus CD4<sup>+</sup> T helper cells were sufficient to eventually eliminate virus. The differences between the two LCMV-strains and effects of neutralising antibodies in immunotherapy remain to be clarified.

#### 4. Vaccines?

Attempts at vaccinating mice with UV-light inactivated or formaldehyde-inactivated LCMV have completely failed [15,37]. Neither neutralising antibody responses nor primed cytotoxic T cell responses have been detected after such immunisations. In contrast, low dose live virus always mediates protection, particularly via induction of specific CD8 T cell responses. As outlined above, virus persists at very low levels [38] after even a low dose infection and maintains a protective memory mediated by CTLs. Peptides or peptides mixed with a adjuvant are able to induce CTL responses. In contrast to live virus infections such peptide-primed mice exhibit protective immune responses only for a limited time period of perhaps up to 50 days dependent on the dose of peptide vaccine use. Also, vaccination with a vaccinia recombinant virus reveals protection during about 30–70 days, dependent on the recombinant virus dose used. But thereafter, protection fades in parallel with the disappearance of measurable activated CTLs. This lack of protection is evident if the challenging infection is initiated strictly peripherally. If, however, the challenging infection is given via i.v. infection, then antigen and virus immediately reaches spleen and lymph nodes and induces within a few hours the quiescent and highly frequent T cells to become effector cells that can eliminate virus rapidly and efficiently [4]. However, a challenging infection usually does not occur via the i.v. route and therefore this model situation may not be helpful for understanding T cell mediated immunoprotection. Systemic spread of virus is usually taken care of by neutralising antibodies which also in LCMV infections are generated and influence virus control importantly. Vaccines that control LCMV via T cells exist in the form of a low dose wild-type virus infection generating both CTLs and eventually neutral-

ising antibody. Priming of CTLs alone is for a brief period of time usually sufficient to control virus efficiently, but such control does not go beyond about 2 months (unless virus persists at low levels [32,39]). For non-cytopathic viruses, this is not of great importance because, as experience shows, transmission occurs before or at birth at a time when the recipient host is immunoincompetent and therefore cannot generate immunopathology [17,40]. Thus, like for TB protection of long duration is achieved for LCMV or HIV by a low level persisting infection that is well controlled by T and B cell immunity for a very long time.

#### 5. Conclusion

HIV is representative of non- or little-cytopathic infectious agents that typically infect the next generation or jump hosts near, at, or right after birth, whose disease causing process involves immunopathology and which is never really eliminated from the host, even under optimal conditions of long-term resistance and lasting immunity. These agents have in common that they often are highly mutable (RNA viruses) that persist in hosts at various levels in various peripheral and lymphatic organs and that can integrate to variable extents (retroviruses, unknown mechanisms for LCMV). Comparable to TB and leprosy, classical parasitic infections (such as malaria, trypanosomiasis and schistosomiasis), such infections are controlled over time by both activated T cell response plus a protective usually neutralising antibody response. Therefore, any vaccine must either keep the effective infectious dose low enough so as to avoid mutations, or alternatively must provide a broad spectrum of potential epitopes so that T cell escape is rendered highly unlikely. Similarly, many serotypes of serovariants must be offered to B cells to generate a broad polyclonal neutralising antibody response, otherwise, escape from neutralising antibodies may occur. Therefore, a vaccine against HIV combines the challenges that we have frustratingly experienced during the past 120 years for TB and leprosy and with the inability of immunologists and microbiologists to develop an efficient vaccine against mycobacteria or against flu. The goals are clear, the tools are theoretically available and therefore there is no a priori reason why eventually a vaccine should not be generated. A realistic goal is not a vaccine that causes sterilising immunity; as shown for TB, leprosy and parasitic infections, this is probably impossible to achieve. The aim must be a vaccine that shifts the overall immunopathological disease kinetics towards later times, by 10, 30 or 60 years. Immunity reducing infection of the mucosa via specific local IgA obviously must be added, but again such IgA vaccines alone will not be sufficient. Last but not least, we must remember that the best vaccine against these non- or low-cytopathic kind of infection is still a physiological low dose infection that is well controlled but persists. Co-evolution of infectious agents and hosts have resulted in an excellently balanced compromise of mutual

benefit, i.e. infection–immunity. So far, we have not come even close to imitate this exquisite balance with “artificial vaccines”; BCG is too attenuated for providing lasting immunity, nef-deleted HIV or SIV vaccines get repaired to become too virulent again. Wild-type TB or wild-type SIV or many parasitic diseases are in fact “very good vaccines” for more than 75% of the natural hosts.

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